Proc. Arthropod. Embryol. Soc. Jpn. 52, 11–14 (2019)

ISSN 1341–1527

[SHORT COMMUNICATION]

Note on the Thickened Serosa and Serosal Cuticle Formed beneath the Embryo in *Scopura montana* Maruyama, 1987 (Insecta, Plecoptera, Scopuridae)

Shodo MTOW and Ryuichiro MACHIDA

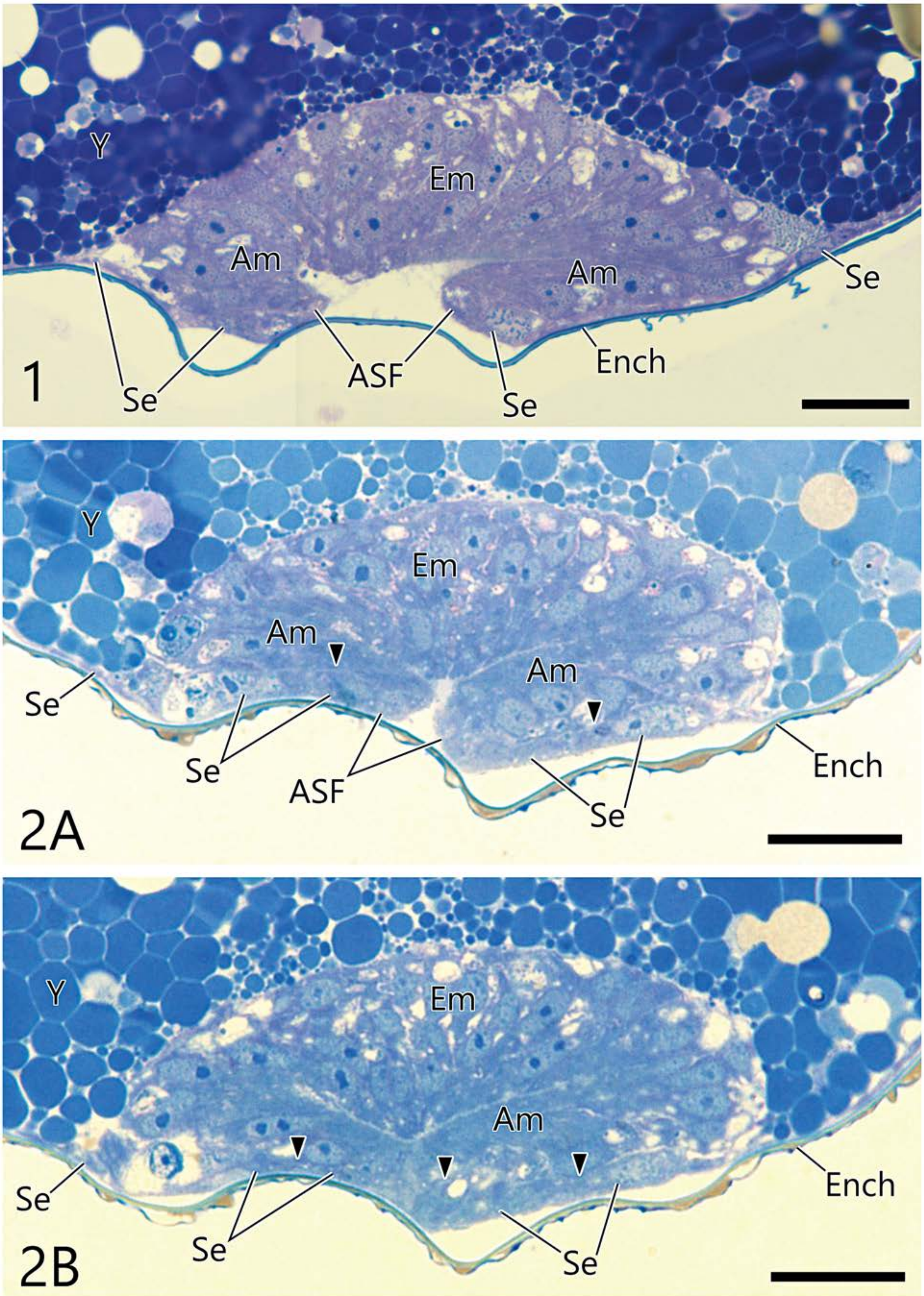
Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305–8572, Japan
Current address: Sugadaira Research Station, Mountain Science Center, University of Tsukuba, Ueda, Nagano 386–2204, Japan
E-mail: s610@sugadaira.tsukuba.ac.jp (SM)

Miller (1939, 1940) described the thickened serosa that forms beneath the embryo in the stonefly *Pteronarcys proteus* Newman, 1838 (Pteronarcyidae), which secretes a bulky cuticular layer. He named the thickened serosa and bulky serosal cuticle the "grumulus" and "grumorium," respectively. Similar serosal structures were reported in plecopteran *Kamimuria tibialis* (Pictet, 1841) (Perlidae) by Kishimoto and Ando (1985), who named the thickened serosa "columnar serosal cells." However, reference to the serosal structures is fragmented, and our knowledge of them is insufficient. For example, there is no information on the thickening of serosa. In our study, we provide a preliminary description of the formation of the thickened serosa and serosal cuticle in the scopurid stonefly, *Scopura montana* Maruyama, 1987 (Scopuridae).

We collected late-instar larvae of *Scopura montana* from the streams in Sugadaira Kogen, Ueda, Nagano, Japan and raised them to the adult stage in the laboratory. The eggs deposited by females were fixed with Karnovsky's fixative (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M HCl-sodium cacodylate buffer, pH 7.2) for 24 h and postfixed with 1% OsO_4 for 1 h. Fixed eggs were dehydrated in a graded ethanol series and embedded in an epoxy resin (Low Viscosity Resin, Agar, Essex, UK). Serial semi-thin sectioning at a thickness of 0.75 μm was performed using an ultra-thin microtome (MT-XL, RMC, Arizona, USA) equipped with a diamond knife (Histo Jumbo Diamond Knife, Diatome, Nidau, Switzerland), according to the methods described by Blumer et al. (2002). Sections were stained using 0.1% toluidine blue solution and examined under a biological microscope (Optiphoto-2, Nikon, Tokyo, Japan).

We followed the embryonic development of *Scopura montana* until the completion of the thickened serosa and serosal cuticle. Soon after a small germ disc of ca. 100 μm in diameter appeared at the posterior pole of the egg, the amnioserosal folds start to form (Mtow and Machida, 2018). During the segregation of the amnion from the embryonic margin, the serosa extends beneath the embryo, causing the formation of amnioserosal folds (Fig. 1). The formation of the amnioserosal folds is more progressive in the posterior region of the embryo than in the anterior region (Fig. 1). The amnioserosal folds draw closer to each other as the amnion progressively segregates and the serosa extends. Figure 2A and B show the embryo with its amnioserosal folds just before their fusion. The inner and outer elements of amnioserosal folds or the amnion and serosa, both of which are massive, are distinguished by a faint boundary between them (arrowheads in Fig. 2A, B). Finally, the amnioserosal folds completely fuse with each other (Fig. 3), and the serosa entirely covers the egg surface. Almost simultaneously, the amnion and serosa of the amnioserosal folds detach from each other. As a result, both amnion and serosa become independent, thick cellular. The embryo and amnion which ventrally covers the embryo or the embryo-amnion composite assumes a compressed, ball-shaped configuration (Fig. 3). Then the serosa, which had been a part of the amnioserosal fold, converges beneath the embryo-amnion composite to form the "thickened serosa," of which cells acquire a radial arrangement in section (Fig. 4). Soon after the fusion of the amnioserosal folds, the serosa has started to secrete the serosal cuticle over the entire surface of the egg (Fig. 4). The serosal cuticle is densely secreted beneath the embryo-amnion composite by the thickened serosa, because the apical surfaces of the thickened serosa have converged there (Fig. 4). As the embryonic development progresses, the embryo gradually elongates and the amnion attenuates (Fig. 5). The serosal cuticle becomes further bloated beneath the thickened serosa to form the "thickened serosal cuticle" (Fig. 5), wherein the three zones that are distinguished: the innermost transparent zone, an intermediate toluidine-philic indented zone, and an outermost yellowish zone.

Our present study revealed that in *Scopura montana*, the thickened serosa that forms beneath the embryo at the posterior pole of the egg is derived from the serosal element of the amnioserosal fold and the thickened serosa heavily deposits a dense, cuticular substance as the thickened serosal

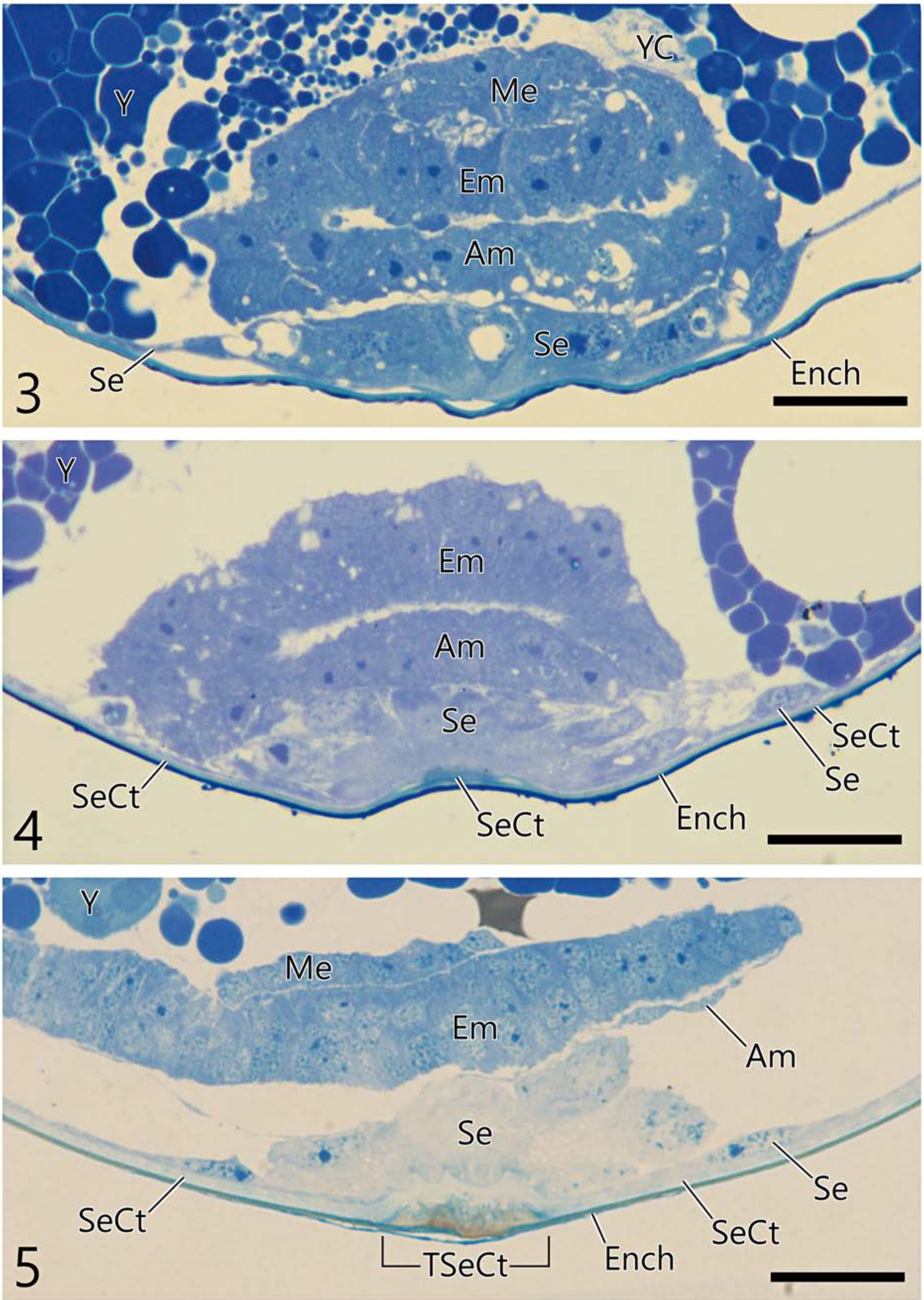


Figs. 1, 2 Sections of a *Scopura montana* embryo undergoing the formation of amnioserosal folds. The exochorion has been removed.

Fig. 1 A sagittal section of an embryo in the early phase of amnioserosal fold formation. Anterior to the left.

Fig. 2 Vertical sections of an embryo with amnioserosal folds just before fusion. A and B show serial sections at a distance of approximately 3 μm. Arrowheads show the boundaries between the amnion and serosa.

Am: amnion, ASF: amnioserosal fold, Em: embryo, Ench: endochorion, Se: serosa, Y: yolk. Scales = 20 μm.



Figs. 3–5 Sections of *Scopura montana* embryos undergoing the formation of a thickening serosa and serosal cuticle. The exochorion has been removed.

Fig. 3 A vertical section of an embryo with the amnioserosal folds that have just fused.

Fig. 4 A vertical section of an embryo with a completely formed thickened serosa.

Fig. 5 A sagittal section of an embryo with a completely secreted thickened serosal cuticle. Anterior to the left.

Am: amnion, Em: embryo, Ench: endochorion, Me: mesoderm, Se: serosa, SeCt: serosal cuticle, TSeCt: thickened serosal cuticle, Y: yolk, YC: yolk cell. Scales = 20 μm.

cuticle. The thickened serosa and thickened serosal cuticle in *S. montana* are apparently comparable to the grumulus and grumorium in *Pteronarcys proteus*, respectively (Miller, 1939, 1940), and the thickened serosa in *S. montana* is comparable to the columnar serosal cells in *Kamimuria tibialis* (Kishimoto and Ando, 1985). Although surveys covering more plecopteran representatives are needed, the thickened serosa and a thickened serosal cuticle may be groundplan features of Plecoptera.

Structures similar to the thickened serosa and/or thickened serosal cuticle have been reported in several polyneopteran lineages, for example, Orthoptera (Slifer, 1938; Slifer and Sekhon, 1963), Grylloblattodea (Uchifune and Machida, 2005), Embioptera (Jintsu and Machida, 2009), and Phasmatodea (Jintsu et al., 2010). Miller (1940) associated the grumulus and grumorium of *P. proteus* with the "hydropylar cells" and "hydropyle(s)" of a grasshopper *Melanoplus differentialis,* respectively (Acrididae), which may enable water uptake by the egg (Slifer, 1938; Slifer and Sekhon, 1963). However, our understanding of these structures in Plecoptera is insufficient to phylogenetically or physiologically compare them with such structures in other polyneopterans. Therefore, more information is needed concerning the development of these structures in Plecoptera.

Acknowledgments: The present study was supported by a Grant-in-Aid from the JSPS, Scientific Research B (JP16H04825) to RM; Research Fellow (JP18J10360) to SM. This is a contribution from the Sugadaira Research Station, Mountain Science Center, University of Tsukuba.

References

Blumer, M.J.E., P. Gahleitner, T. Narzt, C. Handl and B. Ruthensteiner (2002) Ribbons of semithin sections: an advanced method with a new type of diamond knife. Journal of Neuroscience Methods, **120**, 11–16.

Jintsu, Y. and R. Machida (2009) TEM observations of the egg membranes of a webspinner, *Aposthonia japonica* (Okajima) (Insecta: Embioptera). Proceedings of the Arthropodan Embryological Society of Japan, **44**, 19–24.

Jintsu, Y., T. Uchifune and R. Machida (2010). Structural features of eggs of the basal phasmatodean *Timema monikensis* Vickery and Sandoval, 1998 (Insecta: Phasmatodea: Timematidae). Arthropod Systematics and Phylogeny, **68**, 71–78.

Kishimoto, T. and H. Ando (1985) External features of the developing embryo of the stonefly, *Kamimuria tibialis* (Pictet) (Plecoptera, Perlidae). Journal of Morphology, **183**, 311–326.

Miller, A. (1939) The egg and early development of the stonefly, *Pteronarcys proteus* Newman (Plecoptera). Journal of Morphology, **64**, 555–609.

Miller, A. (1940) Embryonic membranes, yolk cells, and morphogenesis of the stonefly *Pteronarcys proteus* Newman (Plecoptera: Pteronarcidae). Annals of the Entomological Society of America, **33**, 437–477.

Mtow, S. and R. Machida (2018) Egg structure and embryonic development of arctoperlarian stoneflies: a comparative embryological study (Plecoptera). Arthropod Systematics and Phylogeny, **76**, 65–86.

Slifer, E.H. (1938) The formation and structure of a special water-absorbing area in the membranes covering the grasshopper egg. Quarterly Journal of Microscopical Science, **80**, 437–457.

Slifer, E.H. and S.S. Sekhon (1963) The fine structure of the membranes which cover the egg of the grasshopper, *Melanoplus differentialis*, with special reference to the hydropyle. Quarterly Journal of Microscopical Science, **104**, 321–334.

Uchifune, T. and R. Machida (2005) Embryonic development of *Galloisiana yuasai* Asahina, with special reference to external morphology (Insecta: Grylloblattodea). Journal of Morphology, **266**, 182–207.